

Bioactive Polymeric Composites Based on Hybrid Amorphous Calcium Phosphates

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INTRODUCTION

AMORPHOUS calcium phosphate (ACP), an important precursor in the biological formation of hydroxyapatite (HAP), has recently been investigated for use as a bioactive filler in resin-based dental materials [1–3]. As its name suggests, ACP is a single phase calcium phosphate salt that lacks the long-range periodic atomic scale order of crystalline materials such as HAP [4]. This internal disorder is also reflected in particles with a spheroidal morphology whose surfaces lack flat, faceted crystalline-like features. On the other hand, ACP has a uniform composition very similar to that of a hydrated tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2 \times \text{H}_2\text{O}$ and a solution ion activity product that is constant over the pH range 7.4 to 9.2. These two features suggest that ACP has a well-defined solubility-controlling local unit. This unit, however, is much more soluble than HAP and, as a result, ACP readily converts into the latter at these pHs. The transformation can be slowed considerably by the inclusion of stabilizing ions such as pyrophosphate (P_2O_7). When used in its P_2O_7 -stabilized form as a filler in polymeric composites, ACP can release supersaturating levels of Ca^{2+} and PO_4^{3-} ions over extended periods of time to form HAP external to the composite [1,2]. In addition, these composites can effectively remineralize *in vitro* caries-like

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enamel lesions that were artificially induced in extracted bovine incisors [3]. ACP composites, however, lack the strength of conventional glass filled dental composites that are widely used in restorative and sealant applications.

The purpose of this study was to determine the feasibility of introducing glass-forming elements during the preparation of ACP so that the resulting hybrid fillers, e.g., silica- and zirconia-modified ACP, would have greater potential for strengthening the composite by improving interfacial interactions with the polymer phase. Specifically, the glass-forming agents tetraethoxysilane [$\text{Si}(\text{OC}_2\text{H}_5)_4$; TEOS], Na_2SiO_3 and zirconyl chloride [ZrOCl_2], were used to modify P_2O_7 -stabilized ACP fillers. New composites containing these hybrid or modified ACP fillers were then evaluated to establish whether introduction of silica- or zirconia-ACP fillers improved their mechanical strength without compromising their remineralization potential.

EXPERIMENTAL

METHACRYLATE RESIN FORMULATIONS

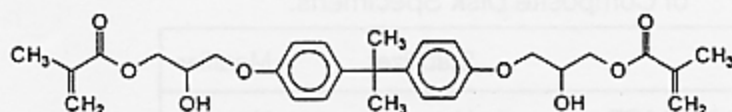
The polymeric phases of the ACP composites were derived from the materials shown in Table 1. The chemical structures of the matrix-forming chemicals are shown in Figure 1.

Two resins differing in degree of hydrophilicity were prepared from Bis-GMA, TEGDMA, HEMA, and ZrM. A photoinitiator system consisting of CQ and 4EDMAB was used to activate resin #1 and resin #2 (Table 2); hereafter designated as R #1 and R #2. R #1, consisting of equal mass fractions of Bis-GMA and TEGDMA, yielded the more hydrophobic composite matrix. Because of the substantial content of HEMA in R #2, the matrix derived from the polymerization of this resin was more hydrophilic than

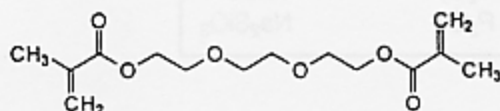
TABLE 1. Monomers and Photoinitiator System Components Employed for Resin Formulations.

Component	Acronym	Manufacturer
2,2-bis[<i>p</i> -(2'-Hydroxy-3'-methacryloxypropoxy)phenyl]propane	Bis-GMA	Freeman Chemical
Triethylene glycol dimethacrylate	TEGDMA	Esstech
2-Hydroxyethyl methacrylate	HEMA	Esstech
Zirconyl dimethacrylate	ZrM	Rohm Tech
Camphorquinone	CQ	Aldrich Chemical
Ethyl 4- <i>N,N</i> -dimethylaminobenzoate	4EDMAB	Aldrich Chemical

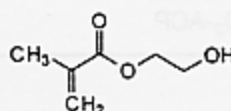
Monomers



Bis-GMA

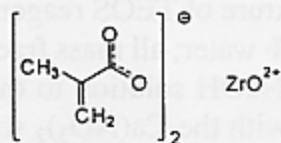


TEGDMA



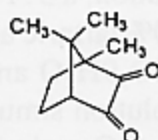
HEMA

Coupling Agent

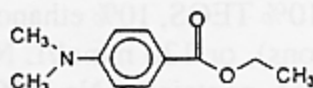


ZrM

Photoinitiator System



CQ



4EDMAB

Figure 1 Chemical structures of the matrix-forming chemicals used in the formulation of composites based on hybrid ACPs.

that derived from R #1. In addition, modest amounts of ZrM were included in R #2 as a dispersing agent for the ACP fillers.

PREPARATION AND CHARACTERIZATION OF THE FILLERS

The various types of fillers used to make the composite disk specimens are given in Table 3. ACP [5] was prepared by rapidly stirring an equal volume of an 800 mmol/L $\text{Ca}(\text{NO}_3)_2$ solution into a 536 mmol/L Na_2HPO_4 solution previously brought to pH 12.5 with 1 mol/L NaOH. The instantaneous precipitation was carried out in a closed system under CO_2 -free N_2 at 22°C. In this way, CO_2 adsorption by the precipitate was minimized.

TABLE 2. Resin Composition (in Mass Fraction as Percent).

Resin	Bis-GMA	TEGDMA	HEMA	ZrM	CQ	4EDMAB
R #1	49.5	49.5	—	—	0.2	0.8
R #2	35.1	35.1	28.0	0.8	0.2	0.8

TABLE 3. Type of ACP Filler Used in the Preparation of Composite Disk Specimens.

Filler	Stabilizer	Modifier
Unstabilized ACP	None	None
P ₂ O ₇ -stabilized ACP	P ₂ O ₇	None
ZrOCl ₂ -ACP	P ₂ O ₇	ZrOCl ₂
TEOS-ACP	P ₂ O ₇	TEOS
SiO ₂ -ACP	P ₂ O ₇	Na ₂ SiO ₃

After the pH stabilized at 10.5 to 11.0, which took less than 5 min, the suspension was centrifuged, the supernatant decanted, and the solid phase washed with ice-cold ammoniated water and then lyophilized. To prepare P₂O₇-stabilized ACP, 10.72 mmol/L of the Na₂HPO₄ was substituted with Na₄P₂O₇ before mixing with the Ca²⁺ component. ZrOCl₂-, TEOS-, and SiO₃-ACP fillers were prepared by adding, respectively, either a 0.25 mol/L ZrOCl₂ solution, a 5:1 volume mixture of TEOS reagent (10% TEOS, 10% ethanol, 10% tartaric acid, and 70% water; all mass fractions), or 120 mmol/L Na₂SiO₃·9H₂O and 1 mol/L NaOH solution to the P₂O₇-containing Na₂HPO₄ solution simultaneously with the Ca(NO₃)₂ solution. ZrOCl₂, TEOS, or Na₂SiO₃ solutions were added to achieve molar ZrOCl₂:Na₂HPO₄, TEOS:Na₂HPO₄ and Na₂SiO₃:Na₂HPO₄ ratios of 0.1, 0.2, and 0.25, respectively.

The amorphous state of the lyophilized solids was verified by powder X-ray diffractometry (Rigaku Denki Co., Ltd., Danvers, MA), and their Ca/PO₄ ratios after dissolution in HCl were determined by atomic absorption (AAS, Perkin Elmer, Norwalk, CT) and UV spectrophotometric [6] (Varian Analytical Instruments, Palo Alto, CA) measurements of Ca²⁺ and PO₄, respectively. Dissolution of the ACP fillers was studied by kinetically following the changes in Ca²⁺ and PO₄³⁻ concentrations in continuously stirred HEPES-buffered (pH = 7.4) solutions adjusted to 240 mOsm/kg with NaCl at 37°C. All solutions initially contained 0.8 mg/mL of the ACP filler.

PREPARATION AND CHARACTERIZATION OF COMPOSITE DISK SPECIMENS

Composite pastes made of the different ACP fillers (mass fraction, 40%) and either R #1 or R #2 (mass fraction, 60%) were formulated by hand spatulation. In some preparations, a silanized BaO-containing glass (7724, Corning glass, mean particle size = 44 μm) was added to the SiO₃-ACP/R #2 formulation to produce pastes that consisted of a mass fraction of 24% SiO₃-ACP, 37% resin, and 38% BaO glass. The BaO glass was

silanized with 0.5% 3-methacryloxypropyltrimethoxysilane (based on the mass of BaO glass according to a previous described method [7]) from cyclohexane using *n*-propylamine as a catalyst. The homogenized pastes were kept under vacuum (2.7 kPa) overnight to eliminate air entrained during mixing. The pastes were molded into disks (15.8 mm to 19.6 mm in diameter and 1.55 mm to 1.81 mm in thickness) by filling the circular openings of flat teflon molds, covering each end of the mold with a mylar film plus a glass slide, and then clamping the assembly together with a spring clip. The disks were photopolymerized by irradiating each face of the mold assembly for 2 min with visible light (Triad 2000, Dentsply International, York, PA). After postcuring at 37°C in air overnight, the intact disks were examined by XRD. Diffraction patterns of the flat surfaces of the disks were recorded at angles of 2θ between 20° and 45° on an X-ray powder diffractometer using graphite-monochromatized CuK α radiation at 40 kV and 40 mA.

DISSOLUTION BEHAVIOR OF THE COMPOSITES

Each individual composite disk specimen was immersed in a 100 mL NaCl solution [HEPES-buffered (pH = 7.4), 240 mOsm/kg, 37°C, continuous magnetic stirring] for up to 264 h. Aliquots were taken at regular time intervals, filtered (Millex GS filter assemblies; Millipore, Bedford, MA), and the filtrates analyzed for Ca²⁺ (AAS) and PO₄ (UV). Upon completion of the immersion tests, the disks were removed, dried, and again characterized by XRD. Variations in the total area of disk surface (*A*) exposed were taken into account and Ca²⁺ and PO₄³⁻ values normalized to an average surface area of 500 mm².

MECHANICAL TESTING OF THE COMPOSITES

The mechanical strength of the composite disk specimens was tested, before and after immersion, under biaxial flexure conditions [8–10] with a universal testing machine (United Calibration Corp., Huntington Beach, CA). The biaxial flexure strength (BFS) of the specimens was calculated according to mathematical expression (1) [8–10]:

$$BFS = AL/t^2 \quad (1)$$

where $A = -[3/4 \pi (X - Y)]$, $X = (1 + \nu) \ln (r_1/r_s)^2 + [(1 - \nu)/2] (r_1/r_s)^2$, $Y = (1 + \nu) [1 + \ln (r_{sc}/r_s)^2]$, and where ν = Poisson's ratio, r_1 = radius of the piston applying the load at the surface of contact, r_{sc} = radius of the support circle, r_s = radius of disk specimen, L = applied load at failure, and t = thickness of disk specimen.

RESULTS AND DISCUSSION

TEOS-modified P_2O_7 -stabilized ACP-filled R #1 disks discharged into buffered saline solution more than three times the amount of both Ca^{2+} and PO_4^{3-} ions than did R #1 disks filled with unmodified P_2O_7 -ACP (Table 4). On the other hand, ion release from $ZrOCl_2$ -modified P_2O_7 -stabilized ACP-filled R #2 disks was somewhat lower than ion release from unmodified

TABLE 4. Ion Release from the Composite Disks Specimens Made of Different Fillers and Resins.

R #1		P ₂ O ₇ -ACP (3)		TEOS-ACP (7)	
Time (h)		Ca ²⁺ (mmol/L) ^a			
24		0.12		0.37	
72		0.23		0.68	
120		0.25		0.78	
264		0.27		0.91	
Time (h)		PO ₄ ³⁻ (mmol/L) ^a			
24		0.08		0.22	
72		0.13		0.39	
120		0.14		0.42	
264		0.15		0.49	

R #2	Unst. ACP (8)	P ₂ O ₇ -ACP (16)	ZrOCl ₂ -ACP (10)	SiO ₃ -ACP (4)	SiO ₃ -ACP + BaOSiO ₂ (4)	
Time (h)		Ca ²⁺ (mmol/L) ^a				
24		0.20	0.36	0.35	0.25	0.17
72		0.29	0.67	0.67	0.49	0.31
120		0.31	0.85	0.81		
168					0.66	0.46
264		0.32	1.02	0.86		
Time (h)		PO ₄ (mmol/L) ^a				
24		0.10	0.21	0.20	0.14	0.10
72		0.13	0.40	0.39	0.28	0.19
120		0.14	0.50	0.47		
168					0.37	0.27
264		0.14	0.61	0.56		

^aConcentration is expressed as mean value for the number of individual runs (indicated in parenthesis next to each filler). Standard uncertainties were: <0.06 mmol/L (Ca^{2+}) and <0.03 mmol/L (PO_4) for P_2O_7 -ACP/R #1; <0.08 mmol/L (Ca^{2+}) and <0.05 mmol/L (PO_4) for TEOS-ACP/R #1; <0.04 mmol/L (Ca^{2+}) and <0.03 mmol/L (PO_4) for unstabilized ACP/R #2; <0.11 mmol/L (Ca^{2+}) and <0.06 mmol/L (PO_4) for P_2O_7 -ACP/R #2; <0.08 mmol/L (Ca^{2+}) and <0.06 mmol/L (PO_4) for $ZrOCl_2$ -ACP/R #2; <0.02 mmol/L (Ca^{2+}) and <0.01 mmol/L (PO_4) for SiO_3 -ACP/R #2; <0.03 mmol/L (Ca^{2+}) and <0.02 mmol/L (PO_4) for SiO_3 -ACP/R #2 + $BaO \cdot SiO_2$ glass.

P₂O₇-stabilized ACP-filled R #2 disks, particularly after time intervals >120 h (Table 4). However, these ion releases were still more than double the release from unmodified ACP composite disks specimens in which unstabilized ACP was used as the inorganic filler. Moreover, ZrOCl₂-modified P₂O₇-stabilized ACP-filled R #2 composite disks reimmersed in fresh saline after an initial 400 h of soaking released almost double the concentration of both mineral ions compared to identically treated unmodified P₂O₇-stabilized ACP-filled R #2 disks. The Na₂SiO₃-modified P₂O₇-stabilized ACP-filled R #2 disks released even lower amounts of Ca²⁺ and PO₄ ions than did the ZrOCl₂-modified disks. The addition of silanized BaO glass suppressed the ion release even further, although the release was still better than that for unstabilized-ACP filled R #2 composite disks.

All ACP-filled composites, both cured and uncured, were stable when kept dry over CaSO₄ in a desiccator. Their XRD patterns (not shown) showed no signs of internal conversion of ACP into HAP. Upon immersion in saline buffer, conversion to HAP occurred more slowly when the modified ACPs were utilized in the polymerized composites. Rapid internal conversion into HAP is not desirable, as the latter phase is not sufficiently soluble to be effective as a remineralizing agent [3]. Additional evidence of the slower internal conversion of ZrOCl₂- and TEOS-modified P₂O₇-stabilized ACP fillers into HAP was obtained from a series of dissolution experiments with the ACP fillers alone in buffered NaCl solutions. It was found that solution Ca/PO₄ molar ratios for suspensions of both modified fillers were much higher (2.05 and 1.75 for ZrOCl₂ and TEOS, respectively) than for suspensions of P₂O₇-stabilized (0.91) or unstabilized (0.55) ACP filler and remained practically constant for 48 h. The drop in the solution Ca/PO₄ molar ratios of the unmodified ACP suspensions is ascribed to the formation of HAP with a Ca/PO₄ molar ratio greater than that of ACP [4]. It is, therefore, concluded that both ZrOCl₂ and TEOS extended the stability of the ACP filler reservoir in the polymer matrix by effectively inhibiting its crystallization into HAP.

However, preliminary studies indicate that solution dynamics may also have an important role in establishing the stability of the ACP component in composites exposed to buffered saline solutions. In these studies, P₂O₇-stabilized R #1 and #2 composites were molded and photopolymerized in situ in 20 mm long × 7 mm wide × 1 mm deep rectangular depressions in flat Teflon plates. When the plates containing the composites were immersed in 40 mL saline solutions for up to 330 h, we found that the ACP in the composite layer adjacent to the back surface of the mold converted more rapidly to HAP than did the ACP in the layer that was in direct contact with the bulk solution (Figure 2). This more rapid conversion suggests that microseepage created a stagnant solution layer between the backside of the composite and the mold. Because ions released from the ACP

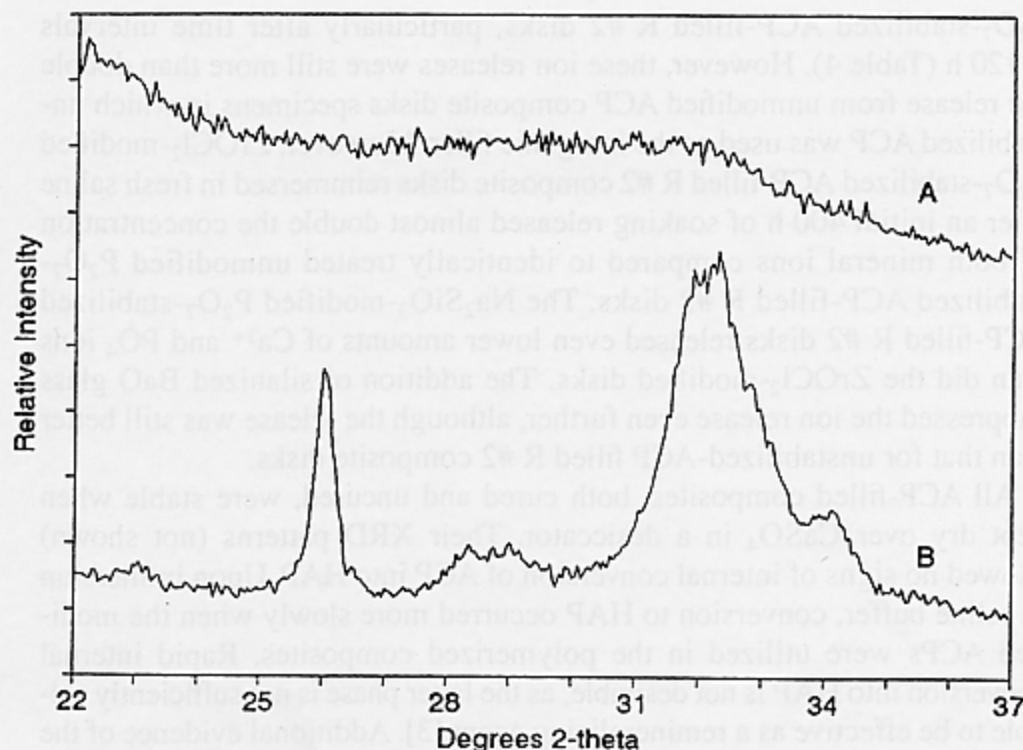


Figure 2 X-ray diffraction patterns of (A) the exposed front surface and (B) the shielded back surface of a P_2O_7 -ACP/R #2 composite slab molded in a Teflon holder and suspended in a HEPES-buffered saline solution for 167 h at 37°C. The relative intensity (RI) values for (A) ranged from 230 to 570 counts/sec and for (B) from 90 to 800 counts/sec. The standard uncertainty of these values is $1/(3RI)^{1/2}$ where 3 is the measurement time constant (in seconds).

could not readily diffuse into the bulk solution from the backside, the layer, once formed, rapidly equilibrated with the ACP. The resulting supersaturation with respect to HAP thus became much higher than in the solution layer adjacent to the front surface of the composite through which the ions could readily diffuse from the ACP into the bulk solution. The stronger thermodynamic driving force resulting from the higher supersaturation would, in turn, favor a more rapid conversion to HAP on the backside of the composite. This finding suggests that the fluid phase inside carious lesions coated with ACP composites may achieve rapid equilibrium with the filler phase. Consequently, a more efficacious remineralization of the lesion may occur than predicted from our ion-releasing solution model.

Results of BFS measurements are summarized in Table 5. In general, composites made of different ACPs had consistently lower mechanical strength than unfilled polymer disks regardless of the resin formulation used. Surprisingly, the mechanical strengths of the dry composites were

TABLE 5. BFS^a of Unfilled and ACP-Filled Composite Disks Before (BFS_{bi}) and After (BFS_{ai}) 264 h Immersion in Buffered Saline Solutions.

Resin	Filler	BFS _{bi} (MPa) ^a	BFS _{ai} (MPa) ^a
R #1	None	140 ± 16 (7)	128 ± 25 (18)
	P ₂ O ₇ -ACP	54 ± 9 (9)	62 ± 12 (13)
	TEOS-ACP	72 ± 17 (9)	75 ± 15 (7)
R #2	None	107 ± 22 (21)	100 ± 15 (7)
	P ₂ O ₇ -ACP	55 ± 19 (11)	51 ± 12 (16)
	ZrOCl ₂ -ACP	69 ± 13 (12)	65 ± 13 (10)
	SiO ₃ -ACP	31 ± 14 (7)	37 ± 8 (6)
	SiO ₃ -ACP + BaO•SiO ₂	57 ± 23 (7)	71 ± 15 (9)

^aBFS values are expressed as mean value ± standard uncertainty. () Indicates number of runs in each experimental group.

not significantly affected after immersion in buffered saline for 264 h. Additionally, comparison of the BFS values of unmodified P₂O₇-ACP composite disks vs. ZrOCl₂ and TEOS modified P₂O₇-ACP composite disks revealed a uniform increase in the mechanical strength of the hybrid ACP composite disks. The relative increase was 25% (55 MPa to 69 MPa) and 27% (51 MPa to 65 MPa) for ZrOCl₂-modified ACP composites and 33% (54 MPa to 72 MPa) and 21% (62 MPa to 75 MPa) for TEOS-modified composites before and after immersion, respectively. The increases were statistically significant ($p < 0.05$, Student's *t*-test) except for TEOS after immersion ($p = 0.22$). The observed improvement in mechanical strength of composites based on ZrOCl₂ and TEOS modified ACP fillers probably resulted from better mechanical integration of such fillers with the polymerized resins. ZrOCl₂ and TEOS possibly changed the ACP particle morphology, intrinsic hardness, and/or surface activity in ways that permitted tighter spatial interlocking with the surrounding matrix, making the composites more resistant to crack propagation.

On the other hand, R #2 based composites containing Na₂SiO₃-modified, P₂O₇-stabilized ACP as a filler were significantly weaker mechanically than unmodified P₂O₇-ACP containing disks (Table 5). The relative decreases in BFS values were 44% and 27% relatively ($p < 0.05$). For reasons that are not clear, Na₂SiO₃, unlike ZrOCl₂ and TEOS appear to weaken the mechanical integration of filler with resin, making the composite less resistant to crack propagation. The observation that BaO glass reversed the negative effect of Na₂SiO₃ on mechanical strength suggests that this material could possibly be a useful co-filler in ACP resin composite applications. Unfortunately, the improvement in strength was offset by lower ion release (Table 4).

CONCLUSION

The results demonstrate that it is possible to improve the mechanical properties of P_2O_7 -ACP filled composites while retaining, if not enhancing, the high remineralization potential of these composites. These improvements were effected by introducing modifying or hybridizing agents, such as TEOS or $ZrOCl_2$, into the ACP component during synthesis. Such modified ACP fillers may be potentially useful for preparing bioactive composites suitable for more demanding restorative, sealant, and adhesive applications. Future studies will focus on the interactions of these modified ACPs with coupling agents and how these surface treatments affect the solubility, ion release, and the strength of these novel bioactive composites.

Disclaimer: certain commercial materials and equipment are identified in this work for adequate definition of the experimental procedures. In no instances does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or that the material and the equipment identified is necessarily the best available for the purpose.

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